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Binding and Degradation of Soluble Immunoglobulin Aggregates by Mouse Mononuclear Phagocytes—Stimulation by Colony-Stimulating Factor¹

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Received February 9, 1982; accepted July 22, 1982

The binding and degradation of soluble guinea pig IgG₂ aggregates by murine mononuclear phagocytes were studied. Bone marrow mononuclear phagocytes cultured in the presence of an embryonic fibroblast-conditioned medium (CM) degraded the aggregates to a much greater degree than did resident peritoneal macrophages. Binding and degradation by resident peritoneal macrophages were enhanced by culture in the presence of CM.

Freshly harvested thioglycollate-induced peritoneal macrophages bound and degraded the aggregates to the same degree as the cultured bone marrow mononuclear phagocytes did. However, the thioglycollate-induced macrophages lost most of these capacities when cultured *in vitro* without CM. When CM was added to these cultures, the capacity to bind and degrade was restored in a dose-dependent fashion. To obtain the maximum effect, exposure to CM must be maintained for more than 2 days. The effect of CM could be reproduced with purified CSF-1. Taken together the results of this study indicate that Fc receptor expression is modulated by CSF-1.

INTRODUCTION

Mononuclear phagocytes are thought to play a crucial role in the handling of immune complexes *in vivo* (1-3). *In vitro* studies have shown that binding, ingestion, and degradation of immune complexes by macrophages are mediated by their Fc γ receptors (4-7). Since mononuclear phagocytes from different sources differ with respect to their state of activation and stage of development (8), their ability to bind and degrade immune aggregates might also differ. To investigate this point, we compared these functions in young and well-defined mononuclear phagocytes, i.e., bone marrow mononuclear phagocytes that proliferate *in vitro* (9, 10), with those in resident and inflammatory macrophages. Because differences were found between these different mononuclear-phagocyte populations, the effects of *in vitro* culture and colony-stimulating factor were further investigated.

¹ Supported in part by the Foundation of Medical Research FUNGO, which is subsidized by The Netherlands Organization for the Advancement of Pure Research (ZWO).

METHODS

Mice

The studies were done in specific pathogen-free male Swiss mice (Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, The Netherlands) weighing between 15 and 20 g.

Cells, Media, and Sera

The techniques used for harvesting and culturing bone marrow cells from the mouse femur in the presence of conditioned medium have been described elsewhere (9). The culture medium was Dulbecco's modified Eagle's medium (Grand Island Biologicals, Grand Island, N.Y.) containing 20% horse serum (Flow Laboratories, Irvine, Scotland) and 20% embryonic mouse fibroblast-conditioned medium (see below). About 1×10^6 nucleated bone marrow cells were incubated in 5 ml culture medium in Teflon culture bags (TCBs) (11). Resident peritoneal macrophages were harvested by lavage of the peritoneal cavity of untreated mice, thioglycollate-induced peritoneal macrophages by lavage 4 days after an intraperitoneal injection of 1.5 ml of Brewer's thioglycollate which was prepared according to the manufacturer's directions (Difco Laboratories, Detroit, Mich.) and stored for at least 4 weeks.

Peritoneal macrophages were cultured in Dulbecco's modified Eagle's medium with 20% horse serum in TCBs, either with or without conditioned medium. All cultures were held at 37°C in a water-saturated atmosphere of 10% CO₂ in air. Resident peritoneal macrophages from normal Hartley guinea pigs were isolated as described elsewhere (12), and were used as control cells in the *in vitro* binding and degradation experiments.

Colony-Stimulating Factor (CSF-1)

The action of CSF-1 (syn.: macrophage growth factor), which is one of the colony-stimulating factors, is virtually restricted to mononuclear phagocyte series (13). Conditioned medium (CM) collected from cultures of embryonic mouse fibroblasts was used as the source of CSF-1, and was prepared according to Goud (14). In short, after dissection of limbs, skull, liver, and spleen, 13 to 15-day-old murine embryos were incubated for 20 min at room temperature in a solution composed of 0.25% trypsin (Difco Laboratories) in phosphate-buffered saline (hemagglutination buffer, Difco Laboratories). After centrifugation (125g) of the cell suspension, the precipitate was suspended in Waymouth medium (MB 752, 1; Gibco) containing 5% newborn calf serum (Gibco). This suspension was divided into 10-ml aliquots, each containing about 3×10^6 embryonic cells, which were incubated in petri dishes (100 mm; Falcon Plastics, Cockeysville, Md.) and held at 37°C in a humidified atmosphere under a constant flow of 10% CO₂ in air. The conditioned medium was collected at regular intervals, usually on the 4th, 8th, and 12th day of incubation, after which new culture medium was added to the dishes. Subcultures were made by trypsinization and replating of the embryonic mouse cells. These subcultures were treated in the same way as the primary cultures. After collection, the conditioned medium was filtered (pore size 0.45 μ m; Millipore S. A. Buc., France) and stored in 5-ml vials at -70°C, unless stated otherwise. In three batches of the conditioned medium, the CSF-1 concentration was measured with a radioimmunoassay (13). These mea-

surements were kindly performed by Dr. E. R. Stanley (Albert Einstein School of Medicine, N.Y.).

Highly purified CSF-1, a gift from Dr. E. R. Stanley was prepared from L-cell-conditioned medium as described elsewhere (13, 15).

Immunoglobulin Aggregates

Monomeric guinea pig IgG₂ was isolated, radiolabeled with ¹²⁵I, and aggregated by incubation at a concentration of 10 mg IgG₂/ml at 63°C for 20 min as described previously (12). Stable aggregates of known size were isolated and identified by ultracentrifugation on calibrated 10 to 30% w/v sucrose gradients containing 0.5% bovine serum albumin for 2 hr at 4°C, with the use of a Spinco SW-41 rotor (Beckmann Instruments Inc., Spinco Div., Palo Alto, Calif.).

Receptors for Guinea Pig IgG₂ on Murine Macrophages

To assess the ability of murine mononuclear phagocytes to bind guinea pig IgG₂, sheep red blood cells (SRBC) covered with guinea pig IgG₂-anti-SRBC in a sub-agglutinating dose were incubated with resident peritoneal mouse macrophages for 60 min at 4°C (12). The percentage of macrophages bearing rosettes of erythrocytes was determined.

Processing of Immunoglobulin Aggregates by Mononuclear Phagocytes

Binding and degradation of immunoglobulin aggregates (AIgG₂) were measured as already described (12). In brief, the mononuclear phagocytes were washed in Tris-buffered Hanks' solution (pH 7.4) containing 0.5% bovine serum albumin (BSA) and pipetted in siliconized glass tubes; per tube, approximately 10⁶ cells were allowed to adhere for 1 hr at 37°C. After vigorous shaking and subsequent aspiration of the nonattached cells the siliconized glass tubes were placed on ice. Subsequently 10 fmol ¹²⁵I-labeled aggregated IgG₂ in 100 µl of TBH-0.5% BSA was added. As a control, the same amount of aggregates were added to glass tubes without cells.

The following were determined:

(a) *Binding.* The adherent cells were incubated with the AIgG₂ at 4°C for 1 or 24 hr. After washing by centrifugation (1500g, 10 min) three times with phosphate-buffered saline (PBS) containing 0.5% BSA, the radioactivity associated with the cells was measured.

(b) *Cell-associated activity.* The tubes with adherent cells and AIgG₂ were transferred to a water bath and incubated under continuous agitation at 37°C. After incubation for 1, 2, and 3 hr, the cells were washed three times by centrifugation (1500g, 10 min) and then radioactivity associated with the cells was measured.

(c) *Degradation of AIgG₂.* The supernatants after centrifugation as under b were combined, 1 ml of 35% trichloroacetic acid (TCA) was added, and after centrifugation (1500g, 10 min) the radioactivity in the pellet was measured.

Binding, cell-associated radioactivity, and degradation were expressed as a percentage of the original input. A correction was made for nonspecific binding of aggregates in control tubes.

RESULTS

More than 95% of the freshly harvested resident peritoneal macrophages incubated with SRBC coated with guinea pig IgG₂ formed rosettes. These results justified the use of guinea pig A IgG₂ for the study of binding and degradation of immune aggregates by mouse macrophages.

Bone marrow mononuclear phagocytes cultured for 10 days in the presence of conditioned medium (BM 10) proved to have the same amount of cell-associated radioactivity (at 37°C) as freshly harvested thioglycollate-induced peritoneal macrophages (TM 0) and less cell-associated radioactivity than resident peritoneal macrophages (RM 0) (Fig. 1). That this reflects a more rapid processing of A IgG₂ by the former two cell types was shown by the degradation experiments. Both bone marrow mononuclear phagocytes and thioglycollate-induced macrophages degraded A IgG₂ more efficiently than resident macrophages (Fig. 1).

Next, the effect of culture on binding and degradation was studied. When thioglycollate-induced macrophages were cultured for 10 days without conditioned medium TM 10, the capacity to bind (Fig. 2A) and degrade A IgG₂ (Fig. 2B) decreased strongly when the cells were cultured (compare TM 0 with TM 10). In another set of experiments it was found that a 50% decrease in the capacity to degrade was already present after 1 day of culture. However, when thioglycollate-induced peritoneal macrophages were cultured in the presence of 20% conditioned medium (TM 10 CM), the capacity to bind (Fig. 2A) and degrade (Fig. 2B) immune aggregates was of the same order as that of freshly harvested thioglycollate-induced cells (TM 0). The degree of binding and degradation in the different experiments varied considerably; this is largely accounted for by the use of two different batches of immune aggregates and of different batches of conditioned medium.

Guinea pig resident macrophages (GPM) bound and degraded the guinea pig

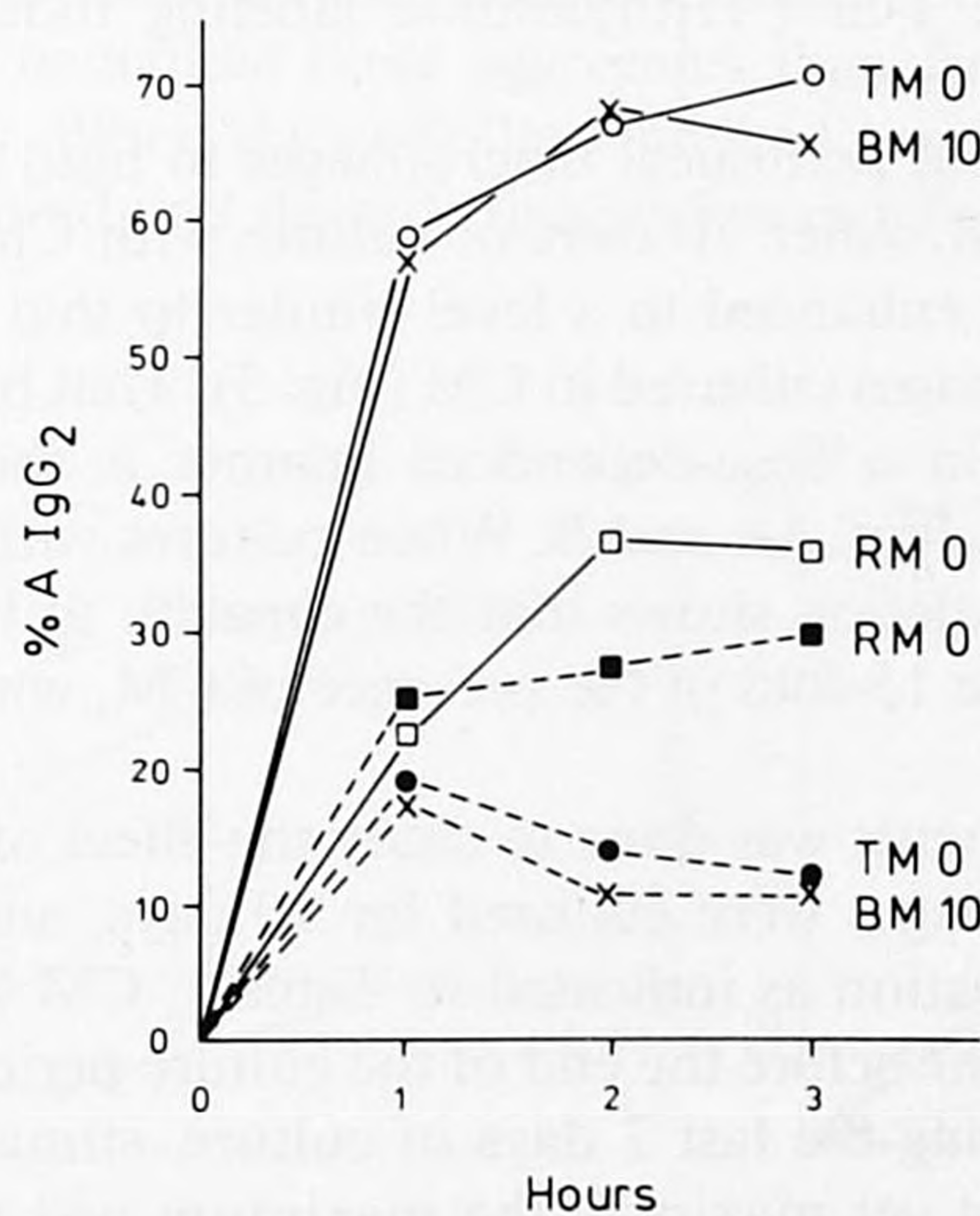


FIG. 1. Immunoglobulin aggregates associated with cells at 37°C (—) and degraded immunoglobulin aggregates (---). Bone marrow mononuclear phagocytes cultured for 10 days in embryonic fibroblast-conditioned medium (BM 10) were compared with freshly harvested thioglycollate-induced peritoneal macrophages (TM 0) and resident peritoneal macrophages (RM 0).

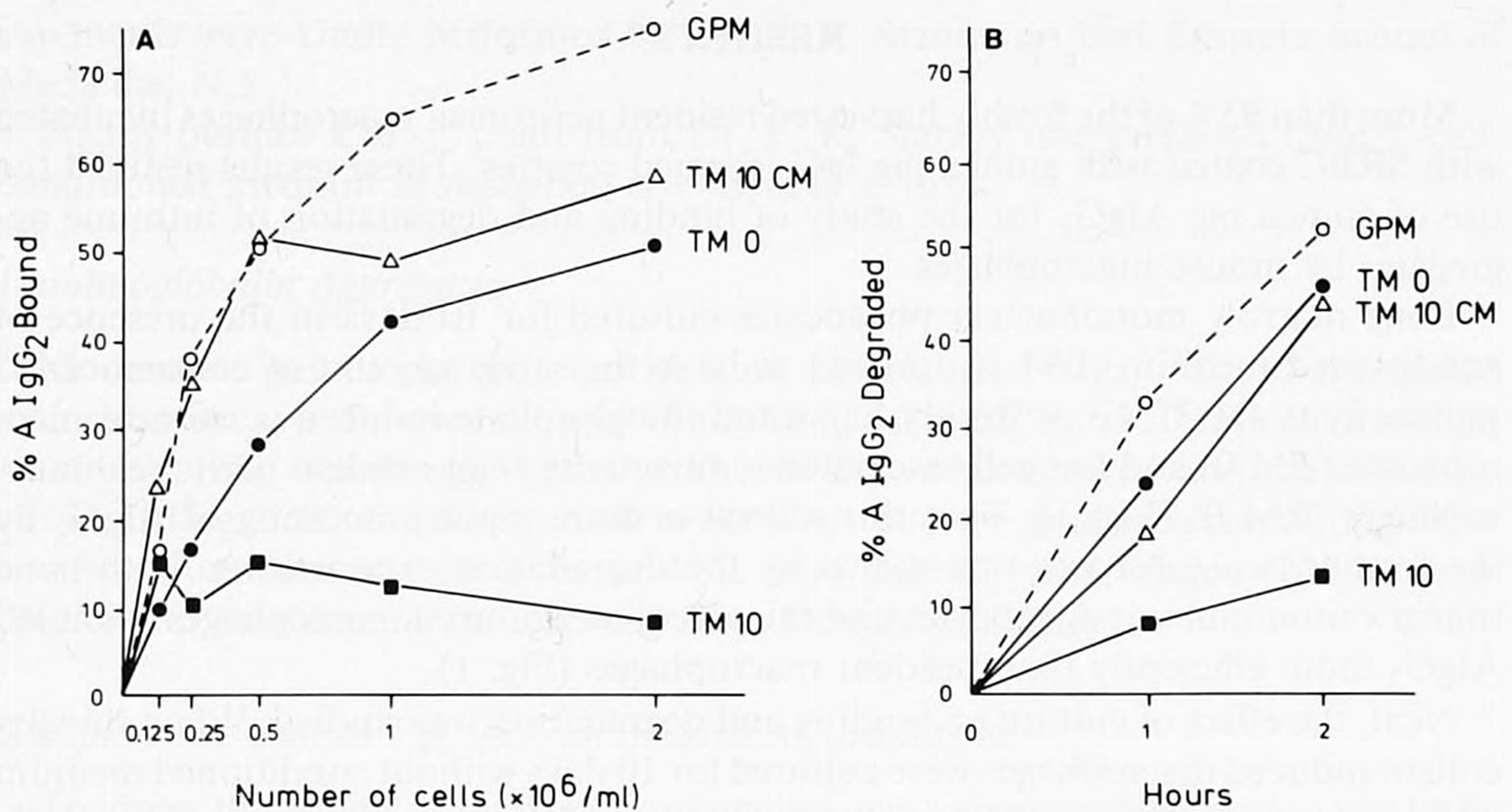


FIG. 2. Binding at 4°C (A) and degradation (B) of soluble immunoglobulin aggregates by noncultured thioglycollate-induced macrophages (TM 0), thioglycollate-induced macrophages cultured for 10 days in the absence of conditioned medium (TM 10), or in its presence (TM 10 CM). The binding and degradation by resident guinea pig macrophages (GPM) are given for comparison. Binding was measured after 24 hr of incubation.

aggregates slightly better than the murine mononuclear phagocytes did (Fig. 2).

The increased binding and degradation shown by thioglycollate cells cultured in the presence of conditioned medium were not the result of cell proliferation; with the Teflon culture system, the strain of mice, and the batches of media and sera used, the number of cells after 10 days of culture is approximately 85% of the original input. The cell number is the same in cultures with and without conditioned medium. Moreover the 1-hr [3 H]thymidine labeling index in these cultures is below 0.5%.

The capacity of resident peritoneal macrophages to bind and degrade aggregates is also influenced by CM. After 10 days of culture with CM, the capacity to bind and degrade A IgG₂ was enhanced to a level similar to that obtained with thioglycollate-induced macrophages cultured in CM (Fig. 3). That binding and degradation are stimulated by CM in a dose-dependent manner is shown for thioglycollate-induced macrophages in Figs. 4A and B. When cultures without CM and with 20% CM are compared, calculation shows that the capacity to bind, for instance, 10% of the aggregates increase 15-fold in the presence of CM, which indicates enhanced Fc-receptor expression.

Next, a set of experiments was done to study the effect of CM in time. Thioglycollate-induced macrophages were cultured for 10 days, and CM was added after various periods of incubation as indicated in Table 1. CM had little effect when it was added later than 24 hr before the end of the culture period. When the cells were incubated with CM during the last 2 days of culture, stimulation of both binding and degradation was not yet maximal; the maximum was reached with 5 days of incubation. The effect of withdrawal of CM during the last 24 hr of culture was studied with bone marrow mononuclear phagocytes. Under these conditions, degradation decreased strongly (Table 1).

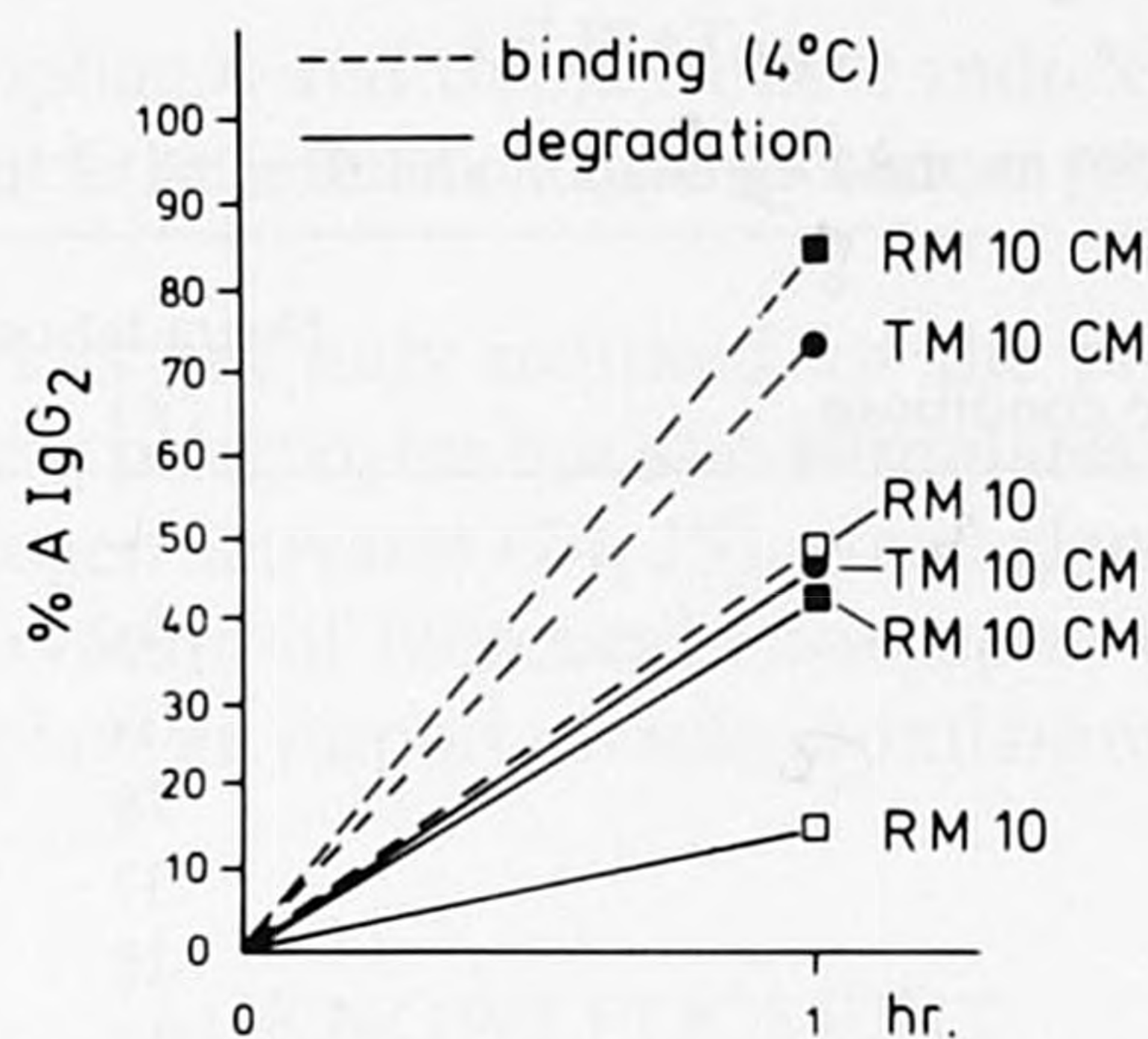


FIG. 3. Binding at 4°C (---) and degradation (—) by resident peritoneal macrophages cultured for 10 days either without conditioned medium (RM 10) or with conditioned medium (RM 10 CM). For comparison, binding and degradation of thioglycollate-induced peritoneal macrophages cultured for 10 days in CM (TM 10 CM) are shown.

To find out whether CSF-1 was responsible for the effects of CM the CSF-1 concentration in the conditioned medium was determined. In three batches of conditioned medium 1730, 1240, and 1620 units CSF-1/ml were detected, which means that on average 150 units CSF-1/ 10^5 cells was added to the cultures. Also highly purified CSF-1 was added to thioglycollate-induced peritoneal macrophages in a concentration of 100 units/ 10^5 cells. This purified CSF preparation stimulated both degradation and binding (Table 1).

DISCUSSION

The present results show that mononuclear phagocytes from bone marrow, maintained in liquid cultures in the presence of embryonic mouse fibroblast-conditioned medium, bind and degrade guinea pig IgG₂ aggregates to about the same degree as freshly harvested thioglycollate-induced peritoneal macrophages and have a considerably greater capacity to handle these aggregates than freshly harvested resident peritoneal macrophages. When thioglycollate-induced macrophages are cultured *in vitro*, their capacity to bind and degrade diminishes rapidly unless CM is added to

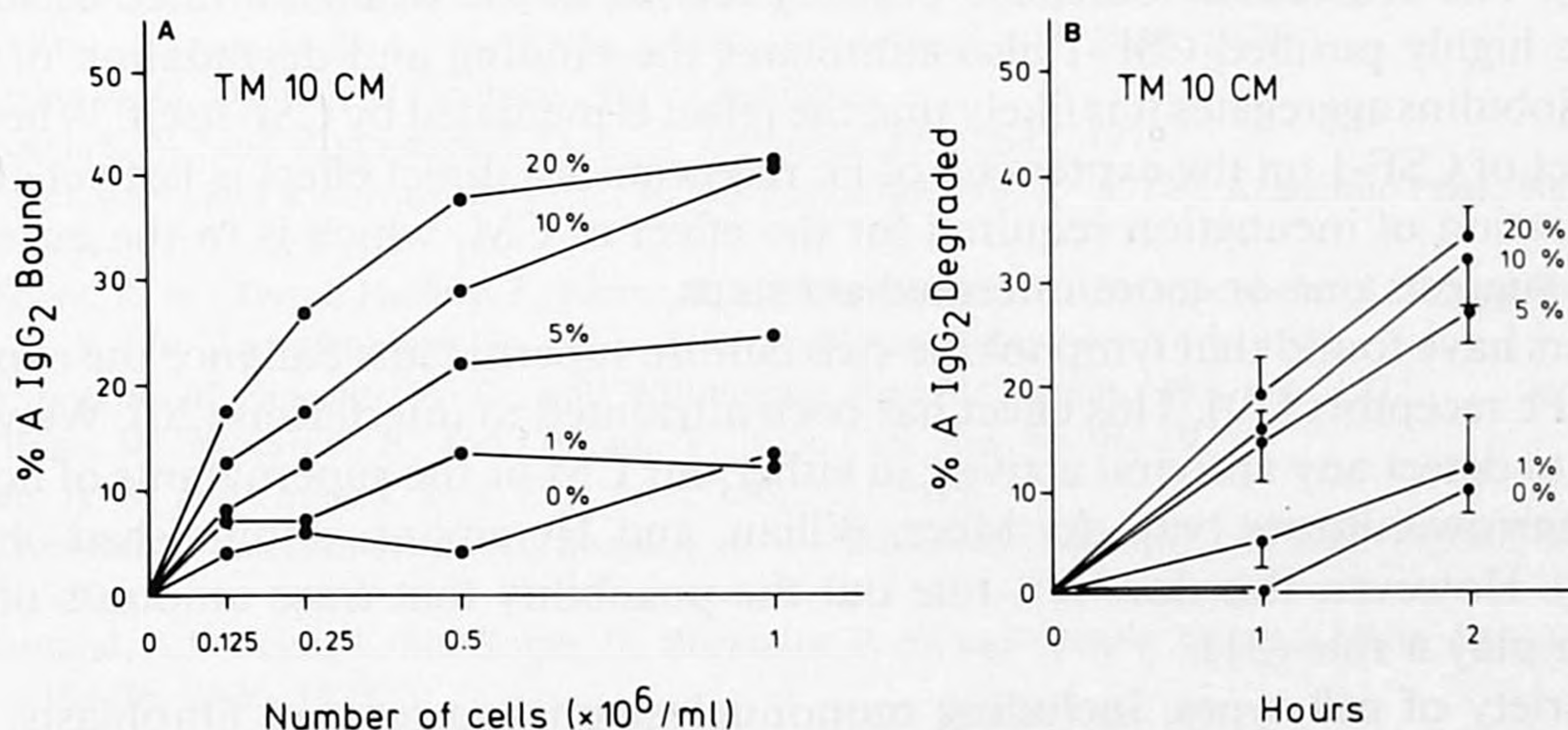


FIG. 4. Binding at 4°C (A) and degradation (B) of immunoglobulin aggregates by thioglycollate-induced cells cultured for 10 days in the presence of various concentrations of conditioned medium.

TABLE 1

Effect of CM and CSF on the Degradation and Binding of Immune Aggregates

Cell type and culture conditions	Degradation (%)	Binding ^a (%)
TM (cultured 10 days)		
CM throughout	100 ^b	100 ^b
CM last 5 days	108	100
CM last 2 days	76	79
CM last 24 hr	37	n.d. ^c
CM last 6 hr	28	n.d.
CM last hr	13	n.d.
No CM	12	n.d.
TM, cultured 10 days with purified CSF-1	56	84
BM, cultured 10 days		
CM 10 days	85	n.d.
No CM last 24 hr	14	n.d.

^a Binding measured after 1 hr of incubation.^b For comparison of sets of experiments, the activity of TM cultured for 10 days with CM was arbitrarily put at 100%.^c Not done.

the culture medium. The activity of resident peritoneal macrophages can also be enhanced by addition of CM to the culture. On the other hand, cultured bone marrow mononuclear phagocytes rapidly lose most of this activity when CM is withdrawn from the culture. Thus, CM must contain one or more substances that stimulate the processing of immunoglobulin aggregates. The stimulation of binding found in this study, and others' findings that attachment is the rate-limiting step in the processing of immunoglobulin aggregates (16–18), suggest that this effect is mainly, if not completely, due to increased Fc-receptor expression in the presence of CM.

In our study, the Fc receptor expression decreased during *in vitro* culture without CM. Others have found an increase in phagocytic capacity of cultured macrophages (19, 20). The differences found are possibly related to the strains of mice used.

Since highly purified CSF-1 also stimulates the binding and degradation of immunoglobulins aggregates it is likely that the effect is mediated by CSF itself. Whether the effect of CSF-1 on the expression of Fc receptors is a direct effect is not yet clear. The duration of incubation required for the effect of CM, which is in the order of 2 days, suggests one or more intermediate steps.

Others have found that lymphokine-rich culture supernatants enhance the expression of Fc receptors (19). This effect has been attributed to interferons (20). We were unable to detect any antiviral activity in either our CM or the supernatants of liquid bone marrow cultures (van der Meer, Billiau, and Heremans, unpublished observations). However, this does not rule out the possibility that trace amounts of interferon play a role (21).

A variety of cell types, including mononuclear phagocytes and fibroblasts, are capable of producing CSF-1. CSF-like substances have been detected in inflammatory exudates (22, 23). Therefore, the differences found between freshly harvested

resident peritoneal macrophages and thioglycollate-induced macrophages might be explained by the exposure of the latter cell type to factors present in the inflammatory exudate.

The finding that CSF-1 is not only required for the proliferation of committed stem cells and mononuclear phagocytes but also stimulates mononuclear phagocytes *in vitro* to secrete plasminogen activator (24, 25), prostaglandins (26), and interleukin I (21) as well as the observation of increased Fc-receptor expression in the present study suggest that CSF-1 plays an important role in inflammation and in the immune response.

ACKNOWLEDGMENT

The authors wish to acknowledge the contributions of Dr. Aize Kylstra to the initial part of the study.

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